# Boat-Shaped Leaf Symptoms of Satsuma Mandarin Associated with Citrus Tristeza Virus (CTV)

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ABSTRACT. The boat-shaped leaf symptom, usually thought to be associated with satsuma dwarf virus (SDV), is commonly observed in satsuma orchards in China. Since 1985, 80 samples from satsuma trees with boat-shaped leaves collected from 9 provinces were tested for SDV infection by inoculation on white sesame and by ELISA. Three out of 80 samples, collected from plants imported from Japan in the 1980s, were infected with SDV and the others were SDV-free. Boat-shaped leaf symptoms of some of the SDV-free samples were graft-transmissible. All of 18 SDV-free samples with boat-shaped leaves reacted positively by ELISA with an antiserum to CTV. More severe boat-shaped leaf symptoms were induced by heavy pruning of suspect trees in spring. Graft-inoculation of CTV into virus-free satsuma showed that CTV could also induce boat-shaped leaf symptoms. We suggest that boat-shaped leaf symptoms of satsuma in China are generally associated with CTV infection.

Boat-shaped leaf symptoms of satsuma mandarin are usually thought to be associated with satsuma dwarf virus (SDV) (10). Miyakawa and Sasaki (8) reported that typical boat-shaped leaf symptoms were found on Yonezawa satsuma without SDV infection as determined by indexing to white sesame and cowpea. Koizumi et al. (7) reported that some Kusumoto satsuma trees with severe dwarf symptoms including small leaves, short internodes, multiple sprouting and beak-tip or boat-shaped leaves were infected with SDV, while others were free of SDV. Their results indicated that Kusumoto satsuma has an inherent ability to develop boat-shaped leaves occasionally and that this characteristic is enhanced by infection with severe CTV. Trees infected with CTV suffered only minor production loss, however, co-infections with SDV and severe CTV cause marked dwarfing which results in heavy production losses.

Since 1985, 80 satsuma samples including 40 cultivars with boat-shaped leaf symptoms were collected from 9 provinces and assayed for SDV infection by the white sesame indicator and by ELISA. These results showed that three of the 80 samples, collected from trees originally imported from Japan in the 1980s, were infected with SDV, while the other 77 were SDV-free (4, and C. Zhou, unpublished data). Boat-shaped leaf symptoms from some of the SDV-free samples were graft-transmissible (13). All of 18 SDV-free samples with boat-shaped leaf symptoms were found to be infected with CTV by ELISA. In May 1988, 1,109 20-year-old satsuma trees including 17 cultivars on trifoliate orange in an orchard located at the Citrus Research Institute, CAAS, Chongqing, were surveyed for boat-shaped leaf symptoms. Boat-shaped leaves were found on 685 trees. Six cultivars were tested by ELISA and all were found infected with CTV. More severe boat-shaped leaf symptoms followed heavy pruning in spring. Therefore, in order to determine if CTV can cause boat-shaped leaf symptoms in satsuma, three CTV isolates were graft-inoculated into satsuma propagated on different rootstocks.

## MATERIALS AND METHODS

**Trial 1.** Eleven 2-yr-old Anliuchen (sweet orange) seedlings and four 3-yr-old satsuma seedlings were

graft-inoculated with two pieces each of CTV-infected stem bark in October, 1991. Ten seedlings of Anliu-chen and six of satsuma were held as non-inoculated controls. A bud of an Owari satsuma seedling was grafted onto each seedling of Anliu-chen as the scion top.

Forty seedlings of 5 varieties including Goutou-chen, small leaf-large flower trifoliate orange, Ponkan mandarin, Wanbai-you pummelo and Jinchen sweet orange were grafted with satsuma buds in March, 1993. For each variety, 8 plants were used. Four of them were graft-inoculated with CTV isolate TR-L514 (11) in 1989 and four served as uninoculated controls.

**Trial 2.** Eight to 12 2-yr-old seedlings of citrange and Goutou-chen were each graft- inoculated with the CT-6 or the CT-9 isolate of CTV, respectively, by the same method described above. Eight seedlings of each variety were held as non-inoculated controls. A bud of an Owari satsuma seedling was propagated onto each seedling as the scion top.

The CT-6 CTV isolate was from Owari satsuma and it caused severe vein clearing, stem pitting and dwarfing in Mexican lime and severe seedling yellows and dwarfing in Eureka lemon and grapefruit. Isolate CT-9, isolated from Miyagawa satsuma, caused moderate vein clearing and stem pitting in Mexican lime, and mild seedling yellows in grapefruit.

Double antibody sandwich (DAS) ELISA was used to assay for CTV and SDV (2, 11, 12). The antibody to CTV and alkaline phosphatase-conjugated antibody were from Sanofi (France). The antibody to SDV and the correspondent conjugate with alkaline phosphatase were provided by the Plant Protection Association Research Institute of Japan. SDV antiserum was prepared in our laboratory in 1994 and used to diagnose SDV by protein-A sandwich(PAS) ELISA (3).

All the seedlings used were held in a screenhouse.

## RESULTS AND DISCUSSION

Plants were observed for 2 to 6 yrs following inoculation. Visual assessments demonstrated that 13 out of 74 plants inoculated with the CTV isolates showed boat-shaped leaf symptoms; whereas the 52 uninoculated control trees remained symptomless (Table 1). All the plants with boat-shaped leaf symptoms were determined by ELISA to be CTV-infected. These result indicated that CTV can also cause boat-shaped leaf symptoms in satsuma.

Since 1987, graft-transmission experiments were conducted using inoculum from trees with boat-shaped leaf symptoms collected from nine provinces. Observation over a period of 6 yrs indicated that 18 out of 176 satsuma plants tested were positive for boat-shaped leaf symptoms and all were infected with CTV. When inoculated with both SDV and CTV, 19 out of 25 plants showed boat-shaped leaf symptoms. These results indicate that CTV can cause the boat-shaped leaf symptoms in satsuma but the incidence of these symptoms 6 yrs after CTV inoculation is much less than that caused by SDV + CTV combined inoculation. Our results differ from reports by Miyakawa (9) and Kishi (6) which indicated that CTV did not cause boat-shaped leaf symptoms in satsuma. These conflicting results may be due to differences in the varieties of satsuma and/or the pathogenic characteristics of the CTV isolates used in both studies.

In addition to SDV and CTV, citrus mosaic virus (CiMV), natsudaidai dwarf virus (NDV) and navel infectious mottling virus (NIMV) can also induce boat-shaped leaf symptoms in satsuma (4). The association of citrus variegation virus (CVV) and citrus tatter-leaf virus (CTLV) with boat-shaped leaves in satsuma was

TABLE 1
RESULTS OF BOAT-SHAPED LEAF SYMPTOM ON SATSUMA INOCULATED WITH CTV

| Inoculation date[y] | Rootstock | Inoculum | No. trees | No. trees with boat-shaped leaves per year[x] | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1992 | 1993 | 1994 | 1995 |
| Oct. 1991 | Anliu-chen | TR-L514 | 11 | 2 | 0 | 4 | 4 |
| | Satsuma | TR-L514 | 4 | 0 | 0 | 0 | 1 |
| Nov. 1989[z] | Goutou-chen | TR-L514 | 4 | | | 0 | 2 |
| | Trifoliate | TR-L514 | 4 | | | 0 | 0 |
| | Ponkan | TR-L514 | 4 | | | 0 | 1 |
| | Wanbai-you | TR-L514 | 4 | | | 0 | 0 |
| | Jin-chen | TR-L514 | 4 | | | 0 | 0 |
| Oct. 1993 | Citrange | CT-6 | 8 | | | 1 | 2 |
| | | CT-6 | 12 | | | 1 | 2 |
| | Goutou-chen | CT-6 | 9 | | | 0 | 0 |
| | | CT-6 | 10 | | | 0 | 1 |

[x]Observations made each year in spring.
[y]52 control plants were symptomless.
[z]Grafted with satsuma bud in March 1993.

previously noticed by C. N. Roistacher and T. Miyagawa, respectively (personal communication).

Therefore, because boat-shaped leaf symptoms may be induced by citrus viruses other than SDV, its presence on satsuma trees cannot be used as the only diagnostic to confirm SDV infection.

CTV is widely distributed in the field in China (1, 5). Therefore, it is suggested that boat-shaped leaf symptoms of satsuma in China are associated with general infection by CTV.

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